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THIS OPINION WAS NOT WRITTEN FOR PUBLICATION

The opinion in support of the decision being entered today
(1) was not written for publication in a law journal and
(2) is not binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte JOHN L. KNOPE
AND JAMES CLARK

Appeal No. 93-3364
Application 07/486,628¹

ON BRIEF

Before WILLIAM F. SMITH, GRON, and PAK, Administrative Patent Judges.

WILLIAM F. SMITH, Administrative Patent Judge.

DECISION ON APPEAL

This is an appeal from the final rejection of claims 18 through 22. Claims 1 through 4, 12, 14, 15 and 17 are pending but have been withdrawn from consideration by the examiner.

¹ Application for patent filed February 28, 1990.

Claim 18 is illustrative of the subject matter on appeal and reads as follows:

18. In a method for identifying an inhibitor of phospholipase A₂ enzyme including the steps of combining a phospholipid, a candidate inhibitor compound, and a phospholipase A₂ enzyme and observing whether said phospholipase A₂ enzyme cleaves said phospholipid and releases fatty acid thereby, the improvement comprising:

said phospholipase A₂ enzyme being characterized by an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence encoded by the DNA sequences of Table I or Table II;

(b) an amino acid sequence encoded by a DNA sequence capable of hybridizing to said DNA sequence of Table I and Table II under stringent or relaxed conditions; and exhibiting phospholipase A₂ activity in the mixed micelle assay; and

(c) an amino acid sequence encoded by an allelic variant of said DNA sequence of Table I or Table II.

The references relied upon by the examiner are:

Johnson et al. (Johnson) 4,917,826 April 17, 1990

Leslie et al. (Leslie), "Properties and purification of an arachidonoyl-hydrolyzing phospholipase A₂ from a macrophage cell line, RAW 264.7", Biochimica et Biophysica Acta 963: 476-492 (1988).

Suggs et al. (Suggs), "Use of synthetic oligonucleotides as hybridization probes: Isolation of cloned cDNA sequences for human β_2 -microglobulin", PNAS 78 (11): 6613-6617 (1981).

Claims 18 through 22 stand rejected under 35 U.S.C.

§ 112, first and second paragraphs, as non-enabled and indefinite and under 35 U.S.C. § 103 as unpatentable over Johnson and Leslie in view of Suggs. We reverse.

BACKGROUND

As explained in the background sections of the present specification and Johnson, phospholipase A₂s are a diverse family of enzymes which play an important role in mammalian metabolism. Phospholipase A₂ (PLA₂) is responsible for the hydrolysis of arachidonic acid-containing phospholipids, thereby providing substrate for the multiple enzymes of the arachidonic acid cascade. The products of the arachidonic cascade are varied and include prostaglandins, thromboxanes, leukotrienes, and other hydroxylated derivatives of arachidonic acid. These products are collectively referred to as "eicosanoids." Overproduction of eicosanoids is responsible for certain systems of disease processes or conditions, including inflammation, erythema, allergic responses, etc. Thus, identification of inhibitors of PLA₂ may lead to useful therapeutic agents for treating or preventing such PLA₂ mediated conditions or symptoms.

Leslie has identified a mammalian PLA₂ in the cytosolic fraction of a mouse macrophage, i.e., monocytic, cell line RAW 264.7. The ability of RAW 264.7 cells to release arachidonic acid in response to inflammatory stimuli suggested these cells had an arachidonoyl-hydrolyzing PLA₂ and would be a useful model to study the properties of the enzyme. This murine PLA₂ was reported to have a calcium dependent specific activity of

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2 $\mu\text{mol}/\text{min}/\text{mg}$, to be resistant to reducing conditions, and to have a molecular weight of about 60 kD under reducing conditions. The PLA_2 from RAW 264.7 cells was inhibited by all detergents tested which precluded an assessment of substrate specificity using a mixed micelle assay. The presence of more than one arachidonoyl-hydrolyzing PLA_2 in the macrophage cytosol could not be ruled out. See, e.g., page 491, left hand column.

SUMMARY OF THE INVENTION

Appellants claim an improved method of identifying a PLA_2 inhibitor characterized by use of a particular mammalian cytosolic PLA_2 (cPLA_2) which has been isolated from both human and murine sources. The human cPLA_2 was purified from the human monocytic cell line U937 and has the predicted amino acid protein sequence encoded by the DNA sequence of Table I of the specification. Appellants' purified human cPLA_2 is resistant to reducing conditions and yields two major bands at about 60 kD and about 110 kD under reducing SDS-PAGE separation. The 110 kD protein has a specific activity of 20 $\mu\text{mol}/\text{min}/\text{mg}$ in a mixed micelle assay. The 60 kD protein was found by appellants to be biologically inactive.

Appellants' murine cPLA_2 was purified from the mouse monocytic cell line RAW 264.7 using analogous methods to those

disclosed for the human cPLA₂. Like the human cPLA₂, the purified murine cPLA₂ showed two major protein bands at about 60 kD and about 110 kD under reducing SDS-PAGE, was resistant to reducing conditions and had a specific activity of about 20 $\mu\text{mol/min/mg}$ associated with the 110 kD protein in a mixed micelle assay. See pages 17-20 of the specification. Appellants posit at page 18 of the specification that:

Due to the lower degree of purity obtained for the reported smaller molecular weight protein, it is likely that the 60 kD protein characterized as PLA₂ by Leslie et al is the contaminant of the 110 kD PLA₂ of this invention and, as such, does not possess PLA₂ activity.

The isolated murine cPLA₂ of this invention is further characterized as having the predicted amino acid protein sequence encoded by the partial cDNA sequence of Table II of the specification.

DISCUSSION

REJECTION I

The examiner contends that claims 18-22:

are indefinite and non-enabled in the recitation of "an allelic variant" in claim 18, step (c) because deletions of different codons from the DNA would result in numerous possible polypeptides which have not been particularly described in the specification. Furthermore, the mutant polypeptides are potentially inoperable because mutations could result in truncated non-functional proteins....(o)ne of ordinary skill in the art could not determine which DNA variant encode "phospholipase A2" without an undue amount of experimentation, in light of the lack of guidelines and the unpredictability of biological function associations with

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specific protein structure. (Examiner's Answer, paragraph bridging pages 4-5, emphasis added)

We agree with appellants that the examiner's position regarding numerous possible polypeptides and truncated non-functional proteins has no applicability to allelic variants as claimed in claim 18(c) when that claim is properly read in light of the specification. Thus, we do not find that the claim is either indefinite or non-enabled.

To the extent this rejection is based upon the second paragraph of 35 U.S.C. § 112, it is well settled that claim language must not be read in a vacuum, but, rather, must be read in light of the supporting specification and prior art. In re Moore, 439 F.2d 1232, 1235, 169 USPQ 236, 238 (CCPA 1971). Here, the specification defines allelic variations at page 21, lines 1-3 as "naturally-occurring base changes in the species population which may or may not result in an amino acid change" (emphasis added). The examiner has not begun to explain why claim 18 when read in light of this specification definition of the questioned term is indefinite.

To the extent the rejection is based upon the first paragraph of 35 U.S.C. § 112 as being non-enabled, the claimed allelic variations, as defined by the specification, do not encompass deletions as urged by the examiner. Furthermore, since the claimed method for identifying inhibitors of PLA₂ enzyme

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activity, by definition, requires a biologically active enzyme, the examiner's reading of the claim to include truncated non-functional proteins is in error. As set forth in In re Dinh-Nguyen, 492 F.2d 856, 858-59, 181 USPQ 46, 48 (CCPA 1974), "It is not a function of the claims to specifically exclude...possible inoperative substances..." (emphasis omitted).

Rejection I is reversed.

REJECTION II

Claims 18 through 22 have been rejected under 35 U.S.C.

§ 103 as unpatentable over Johnson and Leslie in view of Suggs.

The examiner combines Johnson, which discloses a method for identifying a PLA₂ inhibitor using standard laboratory tests, with Leslie, which discloses purification and properties of a 60 kD murine cPLA₂ isolated from cell line RAW 264.7, and Suggs, which discloses the use of synthetic oligonucleotides as probes for isolation of cloned DNA sequences of any purified protein. The crux of the rejection is that it would have been obvious to one of ordinary skill in the art to use the isolated cPLA₂ of Leslie in the assay of Johnson.

Appellants disagree that the cPLA₂ of Leslie is either identical or substantially identical to the claimed PLA₂ based upon differences in (1) physical characteristics, e.g., a molecular

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weight of 67 kD for Leslie's enzyme versus 110 kD for the enzyme of the present invention, and (2) resistance to detergents; i.e., the instant enzyme is active in the presence of detergent, whereas the enzyme of Leslie is inactive. (Appeal Brief, page 12). Appellants also argue that the enzyme of Table I of the specification is exclusively a human enzyme as opposed to the murine enzyme of Leslie.

As we understand the matter, both the examiner and appellant have focused on the purified 67 kD enzyme of Leslie as being the only relevant part of that disclosure. We agree with appellants that the examiner has not properly established that the purified 67 kD cPLA₂ of Leslie is encompassed by claim 18.

The 67 kD murine cPLA₂ of Leslie is clearly not the 110 kD human cPLA₂ encoded by the DNA sequence of Table I of the specification. Nor does it appear that there is a basis on this record to conclude that the partial murine DNA sequence of the 110 kD enzyme of Table II of the specification encodes the Leslie 67 kD enzyme. Thus, the isolated enzyme of Leslie does not meet either requirement of claim 18(a).

As to the requirements of claim 18(b), the first requirement for an amino acid sequence encoded by a DNA sequence "capable of hybridizing said DNA sequence of Table I and Table II under stringent or relaxed conditions" is broad enough to cover

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any amino acid sequence that is capable of hybridizing with the DNA sequence of Table I or Table II of the specification.² This language can reasonably be read to include the nucleotide sequence coding for the 67 kD cPLA₂ of Leslie. However, the 67 kD cPLA₂ of Leslie clearly cannot meet the second requirement of claim 18(b), i.e., "exhibiting phospholipase A₂ activity in the mixed micelle assay" since Leslie explicitly states in the last sentence on page 490:

...the phospholipase A₂ from RAW 264.7 cells was inhibited by all detergents tested, precluding an assessment of substrate specificity using a mixed micelle system.

As to the requirements of claim 18(c), the status of the 67 kD cPLA₂ of Leslie as an allelic variant of the present enzymes is not an issue as developed above in regard to the requirements of claim 18(a).

On this record, we do not find that the examiner has properly established that the purified 67 kD cPLA₂ of Leslie is encompassed by the method of claim 18. Accordingly, rejection II is reversed.

² The use of the phrase "under stringent or relaxed conditions" appears to be essentially meaningless since it is not readily apparent what hybridizing conditions are excluded by this language.

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NEW GROUND OF REJECTION

Under the provisions of 37 CFR § 1.196(b), we make the following new ground of rejection.

Claims 18, 20, 21 and 22 as it depends on claims 18, 20 and 21 are rejected under 35 U.S.C. § 103 as unpatentable over the combined disclosures of Johnson and Leslie.

Like appellants Leslie extracts murine RAW 264.7 monocytes to obtain a cytosolic fraction with PLA₂ activity. Appellants went on to isolate a 110 kD PLA₂ from that cytosolic fraction. Leslie also indicates that phospholipase activity was "exclusively localized" in the crude cytosolic fraction of the RAW 264.7 macrophage cells (page 481, column 2, second paragraph). The cPLA₂ of Leslie eluted as a single peak of activity between molecular weight markers of 67 and 150 kD (Fig. 8A). Furthermore, Leslie shows that incubation of the crude cytosolic fraction with a known PLA₂ inhibitor, BPB, resulted in inhibition of the monocyte derived PLA₂ (page 484 et seq. "Effect of BPB" and Fig. 7).

At the time of the present invention, one of ordinary skill in the art would have found it obvious to use the crude cytosolic extract of Leslie as the source of PLA₂ in the assay of Johnson

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since Leslie discloses that that extract has PLA₂ activity and is useful to determine PLA₂ inhibitor activity and Johnson places no limitation on the source or purity of the PLA₂ used in that procedure. Since the crude extract of Leslie is obtained from the same cell line from which appellants isolated their murine cPLA₂, it is reasonable to shift the burden of going forward to appellants to establish that the crude extract of Leslie does not necessarily contain their 110 kD PLA₂. There is no evidence of record which establishes that the crude extract used by Leslie does not contain the murine 110kD cPLA₂ of the present invention. It is also important to note that the claims on appeal do not require that the PLA₂ used in the procedure be of any particular purity, i.e., the claims do not exclude the use of a crude extract containing the specified PLA₂ such as that of Leslie.

Claims 19 and 22(19) are not included in this new ground of rejection since Leslie does not suggest the 110 kD human cytosolic PLA₂ of claim 19.

OTHER ISSUES

A review of the record shows one reference which may not have been fully considered as possible prior art. The Gronich et

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al. article (J. Biol. Chem. 271: 37-43 (1990)) described in an article co-authored and supplied by appellant (Clark et al., Cell, 65:1043-1051 (1991)) is said to disclose the purification of a 110 kD cPLA₂ from rat kidney with similar chromatographic and biochemical properties to that of the U937 cPLA₂ of the present invention. The examiner and appellants should evaluate this rat enzyme and determine if it is within the scope of enzymes useful in the method of claim 18 due to the broad hybridization standard set forth in claim 18(b).

Any request for reconsideration or modification of this decision by the Board of Patent Appeals and Interferences based upon the the same record must be filed within one month from the date hereof (37 CFR § 1.197).

With respect to the new rejection under 37 CFR § 1.196(b), should appellant elect the alternate option under that rule to prosecute futher before the Primary Examiner by way of amendment or showing of facts, or both, not previously of record, a shortened statutory period for making such response is hereby set to expire two months from the date of this decision.

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No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

AFFIRMED-IN-PART, 37 CFR § 1.196(b)

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